J. Basic Microbiol. **42** (2002) 3, 207–227

Review

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Candida dubliniensis, a new fungal pathogen

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(Received 27 October 2000/Accepted 20 December 2001)

There is a high interest in *Candida* species other than *Candida albicans* because of the rise and the epidemiological shifts in candidiasis. These emerging *Candida* species are favored by the increase of immunocompromised patients and new medical practices, and most oropharyngeal candidiasis are observed in HIV-infected patients. *Candida dubliniensis* is a recently described opportunistic pathogen that is closely related to *C. albicans* but differs from it with respect to epidemiology, certain virulence characteristics, and the ability to develop fluconazole resistance *in vitro*. *C. dubliniensis* has been linked to oral candidiasis in AIDS patients, although it has recently been associated to invasive disease. *C. dubliniensis* shares diagnostic characteristics with *C. albicans*, as germ tube- and chlamydospore-production, and it is generally misclassified as *C. albicans* by standard diagnostic procedures. Several recent studies have attempted to elucidate useful phenotypic and genotypic characteristics for separating both species. A large variety of methods have been developed with the aim of facilitating rapid and accurate identification of this species. These have included differential chromogenic culture media, immunological tests, and enhanced biochemical and enzymatic panels. Chromogenic isolation media, as CHROMagar *Candida*, demonstrate better detection rates than traditional media, and allow the presumptive identification of *C. dubliniensis* by means of colony color (dark-green colonies). API 20 C AUX system is considered a reference method, but ID 32 C strip, and the VITEK 2 ID-YST system correctly identify most *C. dubliniensis* isolates, being the latter the most accurate. Spectroscopic methods, such as Fourier transformed-infrared spectroscopy, offer potential advantages. However, many authors consider that standard methods for differentiation of *Candida* species are time-consuming, often insensitive and can fail to distinguish *C. dubliniensis*. To overcome these drawbacks, molecular tools have been developed to discriminate *C. dubliniensis*, and particularly those based on the polymerase chain reaction. But, molecular tools prove difficult and too complex for routine use in the clinical laboratory setting and new developments are necessary. Although preliminary studies indicate that most strains of *C. dubliniensis* are susceptible to antifungal agents, fluconazole-resistant strains have been detected. Furthermore, fluconazole-resistant strains are easily derived *in vitro*, showing an increased expression of multidrug resistance transporters, as MDR1.

The augmentation of mycoses has been favored by the increased numbers of immunocompromised individuals and species previously not associated with human disease and novel species have been identified as potential pathogens (COLEMAN *et al.* 1998, PONTÓN *et al.* 2000, SANDVEN 2000). A clear paradigm of this phenomenon is *Candida dubliniensis* that was first identified as a new species by SULLIVAN *et al.* (1995) in Dublin, Ireland while performing an epidemiological investigation of oral candidiasis in HIV-infected and AIDS patients in the early 1990s. Some germ tube- and chlamydospore-positive isolates, initially identified as 'atypical' *Candida albicans*, failed to hybridize efficiently with the *C. albicans*-specific DNA fingerprinting probe 27A. Subsequent analysis of these isolates showed that they constituted a distinct species closely related to *C. albicans* (Sullivan *et al.*

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WILEY-VCH Verlag Berlin GmbH, 13086 Berlin, 2002 0233-111X/02/03■■-0207 $ 17.50+.50/0

Table 1
Epidemiology of human colonization and infection by *Candida dubliniensis*

Clinical specimen/Continent	Reference
Oral	
Asia	POLACHECK *et al.* (2000), TAMURA *et al.* (2000)
Australia and Pacific Islands	MCCULLOUGH *et al.* (1995)
Europe	SULLIVAN *et al.* (1993, 1997), BOERLIN *et al.* (1995), PUJOL *et al.* (1997), BIKANDI *et al.* (1998), CEBALLOS *et al.* (1998), ODDS *et al.* (1998), DÍAZ-GUERRA *et al.* (1999), STAIB and MORSCHHÄUSER (1999), VELEGRAKI *et al.* (1999), BADOC *et al.* (2000), GIAMMANCO *et al.* (2000), KURZAI *et al.* (2000), TINTELNOT *et al.* (2000), QUINDÓS *et al.* (2000), WILLIS *et al.* (2000), MOALIC *et al.* (2001)
North America	KIRKPATRICK *et al.* (1998), SALKIN *et al.* (1998), JABRA-RIZK *et al.* (1999), MEILLER *et al.* (1999), BROWN *et al.* (2000), REDDING *et al.* (2001), SEBTI *et al.* (2001)
South America	RODERO *et al.* (1998), SANO *et al.* (2000), ALVES *et al.* (2001)
Vaginal	
Europe	COLEMAN *et al.* (1997a), KURZAI *et al.* (2000), QUINDÓS *et al.* (2000)
Blood	
Australia and Pacific Islands	MARRIOTT *et al.* (2001)
Europe	MEIS *et al.* (2000), SALESA *et al.* (2001)
North America	Brandt *et al.* (2000), Sebti *et al.* (2001)
Other	(feces, urine, respiratory specimens and wounds)
Asia	Polacheck *et al.* (2000), Kamei *et al.* (2000)

1999). Afterwards, *C. dubliniensis* isolates have been identified in a wide range of clinical settings by many different laboratories (Table 1). But probably *C. dubliniensis* has been present in the community for a long time and has been misidentified as *C. albicans*. This species is primarily associated with recurrent episodes of oral candidiasis in AIDS and HIV-infected patients. However, *C. dubliniensis h*as also been implicated in cases of superficial and disseminated candidiasis in patients with and without HIV infection.

The purpose of this article is to review the most recent information available on *C. dubliniensis* and the relevance of this species in human disease. In particular we wish to highlight the advances being made in the development of rapid and accurate tests to allow the discrimination of *C. dubliniensis* from other *Candida* species, especially *C. albicans*.

Epidemiology

C. dubliniensis was originally identified by SULLIVAN *et al.* (1995) in oral specimens from Irish HIV-infected and AIDS patients with recurrent oral candidiasis. Since then this species has been isolated from a wide variety of clinical settings and there have been many recent reports of the identification of this species throughout the world (Table 1). However, the earliest known isolates of *C. dubliniensis* precede the AIDS pandemics and there have been one isolate deposited in the Centraal Bureau voor Schimmelcultures in Holland as *C. albi-*

cans in 1952 (ODDS *et al.* 1998) and another in the British National Collection for Pathogenic Fungi as *Candida stellatoidea* in 1957 (SULLIVAN *et al.* 1995). This emphasizes the problem of misidentification of *C. dubliniensis* isolates due to its phenotypic similarity with *C. albicans* as in two separate retrospective studies on yeast stock collections, approximately 2% of germ tube and chlamydospore-positive isolates originally identified as *C. albicans* have been found to be *C. dubliniensis* (Coleman *et al.* 1997 b, Odds *et al.* 1998). A similar investigation of a collection of oral yeast isolates from HIV-infected individuals originally identified as *C. albicans* revealed that 16.5% were *C. dubliniensis* (Coleman *et al.* 1997 a).

C. dubliniensis has been mainly recovered from the oral cavity of HIV-infected patients with oral candidiasis as mixed cultures with other species of *Candida*, but some studies have shown that between 5 and 10% of HIV-infected patients with oral candidiasis yielded *C. dubliniensis* in pure culture (COLEMAN *et al.* 1993, 1997 a, 1997 b, CEBALLOS *et al.* 1998). MEILLER *et al.* (1999) found that a history of intravenous drug abuse was present in 50% of the *C. dubliniensis* -positive patients, and in their study, *C. dubliniensis* represented 25% of the yeast-positive cultures. The most common clinical manifestations of oral *C. dubliniensis* infection are the erythematous and pseudomembranous forms (COLEMAN *et al.* 1997 a, CEBALLOS *et al.* 1998). Recently, *C. dubliniensis* has also been implicated in an unusual form of linear gingival erythematous candidiasis (VELEGRAKI *et al.* 1999).

This species can cause oral disease in non-HIV-infected persons and be an oral colonizer at low incidence levels in normal healthy individuals. REDDING *et al.* (2001) described a mixed infection of *C. dubliniensis* and *C. albicans* in a patient undergoing head and neck radiation for oral cancer who developed oropharyngeal candidiasis. WILLIS *et al.* (2001) reported that 70% of 414 insulin-using diabetes mellitus patients carried *Candida* species in the oral cavity. In this study, *C. albicans* was the most commonly isolated, followed by *C. dubliniensis*, which was isolated on 64 occasions. Colonization with multiple *Candida* species was common, and *C. dubliniensis* was present in both carriage and disease states. Seven patients without signs of oral disease had *C. dubliniensis* isolated as the sole *Candida* species, while the same species was associated with various forms of oral candidiasis in six patients.

Moreover, isolates of *C. dubliniensis* have been recovered from cases of systemic disease in HIV- and non-HIV-infected patients (PINJON *et al.* 1998, MEIS *et al.* 1999, SALESA *et al.* 2000, MARRIOTT *et al.* 2001, SEBTI *et al.* 2001) and from vaginal, urinary and fecal specimens (SULLIVAN *et al.* 1995, ODDS *et al.* 1998, QUINDÓS *et al.* 2000). MEIS *et al.* (1999) reported three cases of candidemia (*C. dubliniensis*-positive blood cultures) due to this species in HIV-negative patients with chemotherapy-induced immunosuppression and bone marrow transplantation, one patient receiving cytotoxic chemotherapy for relapsed rhabdomyosarcoma and two patients following allogeneic hematopoietic stem cell transplants. POLACHECK *et al.* (2000) described the isolation of five *C. dubliniensis* strains, one of the five isolates was recovered from urine, and while the remaining four were recovered from upper respiratory tract and oral specimens. None of the patients was HIV positive, but all were receiving broad-spectrum antibacterial agents at the time of *C. dubliniensis* isolation. Finally, SALESA *et al.* (2001) have described a case of a *C. dubliniensis* candidemia in a non-neutropenic patient. Although the portal of entry for candidemia was unknown, the authors believed that it might be related to the intravenous use of cocaine by the patient. Two of the patients reported by BRANDT *et al.* (2000) had a history of intravenous drug abuse too. Six *C. dubliniensis* isolates, initially identified as *C. albicans*, have also been isolated from Spanish intravenous drug user (ODDS *et al.* 1998).

The relative clinical importance of *C. dubliniensis* requires further investigation of its epidemiology and virulence, and these studies should be facilitated by the development of reliable identification techniques.

Table 2
Useful phenotypical characteristics for identification of *Candida dubliniensis*

Test name	Reference
Germ tube production in serum	SULLIVAN *et al.* (1995)
Absence of growth at 42–45 ºC	PINJON *et al.* (1998)
Characteristic rough-looking colonies on STAIB agar	STAIB and MORSCHHÄUSER (1999)
Characteristic chlamydospores on STAIB agar	STAIB and MORSCHHÄUSER (1999)
Initial dark green colonies on CHROMagar Candida	KIRPATRICK *et al.* (1998)
Non-fluorescent colonies on methyl-blue SABOURAUD agar	SCHOOFS *et al.* (1997)
Turquoise smooth colonies on Candida ID agar	QUINDÓS *et al.* (2001)
Reduction of tetrazolium salts	VELAGRAKI and LOGOTHETI (1998)
Absence of beta glucosidase activity	BOERLIN *et al.* (1995)
Antigenic differences with *C. albicans* by IFA	BIKANDI *et al.* (1998), MAROT-LEBLOND *et al.* (2000)

Phenotypic characteristics

In the early 1990s, many authors described the recovery of atypical oral *Candida* isolates from HIV-infected patients. These isolates produced germ tube and chlamydospores (features used for definitive *C. albicans* identification), and had a similar antigenicity as *C. albicans* serotype A, but they showed ATB ID32C and API 20C carbohydrate assimilation profiles that not correlated precisely to *C. albicans* or any other yeast species included in these databases. These isolates known now as *C. dubliniensis* are closely related to *C. albicans* (COLEMAN *et al.* 1997 a, SULLIVAN *et al.* 1995) and differentiation between the two species in the clinical laboratory remains difficult (Table 2).

C. dubliniensis produces chlamydospores more readily and abundantly on Rice agar Tween, Tween 80-oxgall-caffeic acid or cornmeal agar than *C. albicans* (SULLIVAN *et al.* 1993, 1997, JABRA-RIZK *et al.* 1999a, KOEHLER *et al.* 1999). A high frequency of chlamydospore formation has been observed in approximately 57% of *C. dubliniensis* isolates and only in approximately 15% of *C. albicans* isolates (SULLIVAN and COLEMAN 1998). However, this has not been shown to be reproducible in some laboratories (SCHOOFS *et al.* 1997). In a recent study with North American *C. dubliniensis* isolates, 70% of them produced abundant chlamydospores; however, 3.6% of *C. albicans* isolates also exhibited a similar phenotype (KIRKPATRICK *et al.* 1998).

STAIB and coworkers (STAIB and MORSCHHÄUSER 1999, STAIB and ARASTEH 2001) have suggested that the ability of *C. dubliniensis* to produce rough colonies and chlamydospores on STAIB agar (Syn. *Guizotia abyssinica* creatinine agar) provided a simple means of differentiating it from its close relative *C. albicans*. On these agar plates, *C. dubliniensis* formed rough colonies due to mycelial growth and produced abundant chlamydospores whereas *C. albicans* grew only in smooth colonies and without chlamydospore formation. In a recent study, AL-MOSAID *et al.* (2001) observed that while none of the *C. albicans* isolates produced chlamydospores on either STAIB agar or Caffeic acid-ferric citrate agar, more than 84% of the *C. dubliniensis* isolates produced chlamydospores on both of them. All of the *C. albicans* isolates grew as smooth, shiny colonies on STAIB agar after 48–72 h at 30 ºC, while most *C. dubliniensis* isolates grew as rough colonies, many (approximately 65%) with a hyphal fringes. In contrast, most *C. albicans* and *C. dubliniensis* isolates grew as rough colonies on Caffeic acid-ferric citrate agar. For these authors (AL-MOSAID *et al.* 2001), discrimination between these two species on STAIB agar was best achieved on the basis of colony morphology rather than chlamydospore production.

C. dubliniensis isolates (SULLIVAN *et al.* 1995, SULLIVAN and COLEMAN 1997, 1998) grow well at 30–37 °C, as creamy white colonies on solid media, such as Glucose SABOURAUD agar or Potato dextrose agar, but these colonies are indistinguishable from those of *C. albicans*. However, unlike *C. albicans* colonies, *C. dubliniensis* do not grow or grow with difficulty at 45 °C (COLEMAN *et al.* 1997a, 1997b, PINJON *et al.* 1998, SULLIVAN *et al.* 1997b, 1998, JABRA-RIZK *et al.* 1999a, MORSHHÄUSER *et al.* 1999). PINJON *et al.* (1998) found that only 1% *C. albicans* isolates failed to grow at 45 °C. However, KIRKPATRICK *et al.* (1998) observed that 36% *C. albicans* isolates did not grow at this temperature. This discrepancy could be a reflection of the inaccuracy of temperature readings and heat distribution in many laboratory incubators (SULLIVAN *et al.* 1999). This absence or restriction of growth is a simple and economic marker to demonstrate for *C. dubliniensis*, with the drawback that some atypical *C. albicans* isolates share the same characteristic (TINTELNOT *et al.* 2000).

A rapid and inexpensive way to presumptively differentiate between the two species after a previous germ tube test derives from the ability of *C. dubliniensis* to reduce the tetrazolium salt (VELEGRAKI and LOGOTHETI 1998). But TINTELNOT *et al.* (2000) have pointed out the limitations of a screening test based on the reduction in 2,3,5-triphenyltetrazolium chloride by *C. dubliniensis* and not by *C. albicans*, because this reduction is frequently seen with *C. tropicalis* isolates.

Although it is impossible to distinguish between *C. albicans* and *C. dubliniensis* colonies in conventional solid media, the introduction in the clinical microbiology laboratories of chromogenic agars, as CHROMagar Candida (CHROMAGAR Company, France) or Candida ID (BIOMÉRIEUX, France), has proven to be helpful in the identification of *C. dubliniensis* isolates, particularly following primary culture from clinical specimens. While *C. albicans* colonies are a light blue green color on CHROMAGAR Candida, *C. dubliniensis* colonies are a much darker green (particularly pronounced after 48 h) (SCHOOFS *et al.* 1997, SULLIVAN and COLEMAN 1998, KOEHLER *et al.* 1999). CHROMagar Candida has been reformulated by BECTON DICKINSON (USA) and JABRA-RIZK *et al.* (2001) have observed on this reformulated agar lighter green colonies with *C. albicans* that made easier to differentiate between this species and *C. dubliniensis*. However, other authors (SCHOOFS *et al.* 1997, TINTELNOT *et al.* 2000) have reported that the ability of *C. dubliniensis* to produce dark green colonies can be lost with subculture and storage. For TINTELNOT *et al.* (2000) the color of the colonies on CHROMAGAR Candida proved to be insufficient for selecting this species, since only 30 of 53 proven *C. dubliniensis* isolates showed a dark green color in primary cultures. The reasons for this apparent colony color instability has yet to be elucidated but could be related to the higher frequency phenotype switching of *C. dubliniensis* isolates than *C. albicans* ones observed by HANNULA *et al.* (2000).

Recently, ODDS and DAVIDSON (2000) examined the color of colonies of nine *Candida* species on CHROMagar Candida incubated for 24–72 h at 25 °C, 30 °C or 37 °C. Colors and colony forms characteristic of *C. albicans* and *C. dubliniensis* were formed most rapidly and were best differentiated at 37 °C. They concluded that incubation of this chromogenic medium at temperatures below 30 °C is not reliable for presumptive identification of *Candida* sp.

Other culture media, as Methyl blue-SABOURAUD agar (SCHOOF *et al.* 1997) or Candida ID agar (QUINDÓS *et al.* 2001), have been proposed for discriminating *C. dubliniensis* from *C. albicans*. On Methyl blue-SABOURAUD agar, *C. albicans* isolates produced yellow fluorescence when exposed to long-wave ultraviolet light, whereas *C. dubliniensis* isolates did not produce fluorescence. Unfortunately fluorescence was not visible in all *C. albicans* isolates when recovered from storage or repeated subcultures (SULLIVAN *et al.* 1998). On Candida ID agar, most *C. dubliniensis* isolates showed clear turquoise blue colonies, in contrast to *C. albicans* deep blue colonies. However, a small number of *C. dubliniensis* isolates grew as white colonies complicating their identification on this agar medium (QUINDÓS *et al.* 2001).

Table 3
Identification of *Candida dubliniensis* with commercial yeast identification systems (reading at 48 H)

Test name	Identification	Reference
API 20C AUX	Good	GALES *et al.* (1999), PINCUS *et al.* (1999)
ATB ID 32C	Excellent	PINCUS *et al.* (1999), TINTELNOT *et al.* (2000), MOALIC *et al.* (2001)
RapidID Yeast Plus	Good	PINCUS *et al.* (1999)
Vitek YBC	Good	GALES *et al.* (1999), PINCUS *et al.* (1999)
VITEK 2 ID-YST card	Excellent	PINCUS *et al.* (1999), MOALIC *et al.* (2001)

From the earliest observations, *C. dubliniensis* has revealed significant differences with *C. albicans* on comparative analysis of substrate assimilation profiles using ATB ID 32C (BIOMÉRIEUX) and API 20C AUX systems (BIOMÉRIEUX) (PELTROCHE-LLACSAHUANGA *et al.* 1999, SULLIVAN *et al.* 1999). *C. dubliniensis* is unable to assimilate alpha-methyl-D-glucoside, lactate or xylose in contrast to the great majority of *C. albicans* isolates (SULLIVAN *et al.* 1995, KIRKPATRICK *et al.* 1998, SALKIN *et al.* 1998). Moreover, *C. dubliniensis* grows more slowly than *C. albicans* when trehalose is used as the only source of carbon in culture medium. These differences in *C. dubliniensis* carbohydrate assimilation profiles have been included in the databases of ATB ID 32C and API 20C AUX. *C. dubliniensis* can also be accurately identified using a variety of other commercially available yeast identification techniques, including the Auxacolor (BIORAD, USA), API Candida (BIOMÉRIEUX), RapID Yeast Plus (REMEL, USA), VITEK YBC and VITEK 2 ID-YST (bioMérieux) systems (COLEMAN *et al.* 1993, GUTIÉRREZ *et al.* 1994a, 1994b, GALES *et al.* 1999, SULLIVAN *et al.* 1999, WARREN and HAZEN 1999) (Table 3).

Two recent studies (GALES *et al.* 1999, PINCUS *et al.* 1999) have evaluated the usefulness of commercial systems for *C. dubliniensis* identification. GALES *et al.* (1999) studied the inability of this species to utilize xylose and alpha-methyl-D-glucoside by API 20C AUX and VITEK YBC systems and to grow poorly or not at all at 45 °C as identification markers for *C. dubliniensis*. The authors observed that none of the *C. dubliniensis* isolates grew at 45 °C, and 23% *C. albicans* isolates exhibited poor or no growth at this temperature. The xylose and alpha-methyl-D-glucoside tests contained within the API 20C AUX system were both negative for all *C. dubliniensis* isolates and were positive for 98 % (xylose) and 56% (alpha-methyl-D-glucoside) of *C. albicans* isolates. With the VITEK system, 97% *C. dubliniensis* isolates were xylose negative and 95% were alpha-methyl-D-glucoside negative. Conversely, 96% *C. albicans* isolates were xylose positive and 100% were alpha-methyl-D-glucoside positive. The authors concluded that lack of growth at 45 °C and a negative xylose test with either the API 20C AUX or VITEK yeast identification system could be used to provide a presumptive identification of *C. dubliniensis*, but a negative alpha-methyl-D-glucoside test result may misclassify *C. albicans* as *C. dubliniensis*, especially when the API 20C AUX system is used.

PINCUS *et al.* (1999), using similar and other commercial kits, evaluated the utility of assimilation of glycerol, lactate, alpha-methyl-D-glucoside, D-trehalose, and D-xylose by isolates of *C. dubliniensis* and *C. albicans*. At 48 h the assimilation of four carbohydrates in the API 20C AUX system could be used to distinguish them, i.e., glycerol (88 *vs.* 14%), xylose (0 *vs.* 88%), alpha-methyl-D-glucoside (0 *vs.* 85%), and trehalose (15 *vs.* 97%). Similarly, results with the ID 32 C system at 48 h showed that xylose (0 *vs.* 98%), alpha-methyl-D-glucoside (0 *vs.* 98%), lactate (0 *vs.* 96%), and trehalose (30 *vs.* 96%) could be used to separate both species. Phosphatase (9 *vs.* 76%) and alpha-D-glucosidase (23 *vs.* 94%) proved to be the most useful for separation of the species in the RapID Yeast Plus system. While at 24 h the profiles obtained with the VITEK YBC system showed that alpha-methyl-

D-glucoside (10 *vs.* 95%), xylose (0 *vs.* 95%), and glycerol (26 *vs.* 80%) could be used to separate the two species, at 48 h only xylose (6 *vs.* 95%) is useful to separate the two species. The most useful substrates in the VITEK 2 ID-YST system were trehalose (1 *vs.* 89%), alpha-methyl-D-glucoside (1 *vs.* 99%), lactate (4 *vs.* 98%), and phosphatase (83 *vs.* 1%). The authors underlined that the assimilation of alpha-methyl-D-glucoside, trehalose, and xylose proved to be the most useful for species differentiation by the majority of commercial systems. However, KURZAI *et al.* (2000) studying carbohydrate assimilation patterns for *C. dubliniensis* with a novel automated system observed that, in contrast to the previous reports, *C. dubliniensis* is able to utilize D-xylose and trehalose.

Another important difference between *C. dubliniensis* and *C. albicans* isolates is that the former cannot express beta-glucosidase activity. This feature was originally identified by multilocus enzyme electrophoresis and has been the basis of a reliable assay for the discrimination of both species (BOERLIN *et al.* 1995). It was observed that, in contrast with *C. albicans*, *C. dubliniensis* isolates did not produce beta-glucosidase activity. This led to the design of a simple method to differentiate between the two species based on the ability of *C. albicans* to generate fluorescence in the presence of methyl-umbelliferyl-labelled beta-glucoside. This assay has been used to discriminate between *C. dubliniensis* and *C. albicans* in isolates from stock cultures (SULLIVAN *et al.* 1993, 1997, SULLIVAN and COLEMAN 1998), although in a recent analysis of an archival stock collection 67 of 537 (12.5%) *C. albicans* isolates were found to be beta-glucosidase negative (Odds *et al.* 1998).

C. dubliniensis cells grown at 37 °C on SABOURAUD dextrose agar have the ability to coaggregate *in vitro* with cells of the oral bacterium *Fusobacterium nucleatum* (JABRA-RISZK *et al.* 1999b, 1999c). *C. albicans* cells fail to coaggregate with this species at this temperature. These authors have developed a rapid, specific and inexpensive test using the strain ATCC 49256 of *F. nucleatum* to distinguish *C. dubliniensis* from *C. albicans* based on this phenomenon (JABRA-RIZK *et al.* 1999b, 1999c, BROWN *et al.* 2000).

BIKANDI *et al.* (1998) have developed a *C. dubliniensis*-specific antiserum adsorbed with *C. albicans* blastospores. This antiserum reacted with the blastospores of *C. dubliniensis*, but not with *C. albicans* blastospores. In a blind trial using an indirect immunofluorescence assay, the antiserum correctly discriminated between 83 *C. dubliniensis* and 43 *C. albicans* isolates. This test is very rapid and specific; however, its use is limited by the availability of the antiserum and by the immunofluorescence microscopy technique. A similar approach has been suggested by MAROT-LEBLOND *et al.* (2000) that have used hydrophobic components of the germ tube of the dimorphic pathogenic fungus *C. albicans* as immunogens to prepare monoclonal antibodies. Monoclonal antibody 16B1-F10 was shown to be specific to the surface of the mycelium phase of the *C. albicans* and may be a good candidate for use in immunological tests for the rapid differentiation of *C. albicans* and *C. dubliniensis*. The antigenic difference between both species (BIKANDI *et al.* 1998, MORAGUES *et al.* 2001) has been used for the diagnosis of a *C. dubliniensis* candidemia in an intravenous cocaine user detecting specific anti-*C. dubliniensis* antibodies by an indirect immunofluorescence and an immunoblotting assays (SALESA *et al.* 2000).

Other tests which allow the discrimination of *C. dubliniensis* and *C. albicans* include pyrolysis mass spectrometry and Fourier transform infrared spectroscopy (Timmins *et al.* 1998, TINTELNOT *et al.* 2000) which has shown a great reliability among the phenotypic methods.

Finally, we should mention the method developed by PELTROCHE-LLACSAHUANGA *et al.* (2000a) to discriminate between these two closely related yeast species by fatty acid methyl ester analysis using gas-liquid chromatography (Sherlock Microbial Identification System; MIDI Inc., USA). Although the chromatograms of these two species revealed no obvious differences when applying fatty acid methyl ester analysis, a new library (CADLIB) was created by the authors using SHERLOCK Library Generation Software (MIDI). Using this method, only 9.4% isolates of *C. albicans* were misidentified as *C. dubliniensis*, whereas all

isolates of *C. dubliniensis* were correctly identified. Resulting differentiation accuracy was 90.6% for *C. albicans* and 100% for *C. dubliniensis*. However, the technology required to perform these techniques is not widely available at clinical diagnostic laboratories.

Genotypic characteristics

The identification methods based on phenotypic criteria may be subject to variable expression and lead to incorrect identification of isolates. A potentially more stable identification would be one based on the analysis of genetic variability. Genetic methods for *Candida* sp. Identification are currently limited to research usage and to date commercial kits have not become available. There are to traditional approaches for identification based on genetic variation, analyses of electrophoretic karyotype differences and restriction fragment length polymorphisms using gel electrophoresis or DNA-DNA hybridization (LOCKHART *et al.* 2001). Electrophoretic karyotype has progressed with the development of pulse field electrophoresis, which resolves DNA fragments over 50 kb by subjecting the DNA to an electrophoretic field that alternates in its direction (WILLIAMS and LEWIS 2000).

The first *C. dubliniensis* isolates were distinguished from *C. albicans* isolates because of their unusual DNA fingerprint patterns generated using the *C. albicans*-specific DNA fingerprinting probe 27A (SULLIVAN *et al.* 1993, 1995, 1996). The *C. albicans*-specific DNA fingerprinting probe 27A gives a classical fingerprinting pattern of 10–15 strongly hybridizing bands (with *Eco*R1-digested DNA) for *C. albicans*. On the contrary, the patterns of *C. dubliniensis* isolates comprised fewer and weaker bands (SULLIVAN *et al.* 1993, 1995, 1997b, 1998). Following pulsed-field gel electrophoresis of *C. dubliniensis* DNA, ten or more chromosome-sized DNA bands can be resolved, usually with one or more bands less than 1 Mb in size (SULLIVAN *et al.* 1995, ANTHONY *et al.* 1995). In contrast, seven or eight bands are usually resolved in *C. albicans*.

The significant differences in the chromosomal arrangement of sequences in each species has been confirmed using a wide range of DNA profiling techniques, including fingerprinting with oligonucleotides homologous to microsatellite sequences, pulsed-field gel electrophoresis and specific or randomly amplified polymorphic DNA (RAPD) polymerase chain reaction (PCR) analysis (SULLIVAN *et al.* 1993, 1995, RUHNKE *et al.* 1997, 1999, BENNET *et al.* 1998, METZGAR *et al.* 1998, DIAZ-GUERRA *et al.* 1999, IROBI *et al.* 1999, MORSCHHÄUSER *et al.* 1999, ALONSO-VARGAS *et al.* 2000, FUJITA *et al.* 2000, TAMURA *et al.* 2000) (Fig. 1). Karyotyping is time consuming, requiring approximately five days for DNA extraction and pulse field gel electrophoresis and costly specialized equipment for the latter, and only a limited number of samples can be included on each gel. These practical drawbacks limit the value of these methods as diagnostic tools in routine diagnostic microbiology laboratories (WILLIAMS and LEWIS 2000).

A quantitative measurement of the precise phylogenetic relationship between *C. dubliniensis* and *C. albicans h*as been determined by comparing the evolutionarily conserved genes, as those encoding the ribosomal RNA (rRNA) (BOUCHER *et al.* 1996, HAYNES and WESTERNENG 1996). Alignment of gene sequences from the V3 region of the large rRNA subunit and from the entire small rRNA subunit with the corresponding sequences from different *Candida* species demonstrated that *C. dubliniensis* isolates belonged to a separate taxon (SULLIVAN *et al.* 1995, GILFILLAN *et al.* 1998). Sullivan *et al.* (1995) found that a 600 bp region encompassing the V3 variable region of the large rRNA (lrRNA) genes of *C. dubliniensis* and *C. albicans* differed by 2.3%. Similar analysis of the D1/D2 region of the lrRNA genes of both species also revealed a significant degree of nucleotide divergence (MANNARELLI and KURTZMAN 1998). In addition, comparison of the sequence of the self-splicing group I introns present in the lrRNA genes of both species revealed that the *C. dubliniensis* intron is almost identical to that of *C. albicans* except for two widely diver-

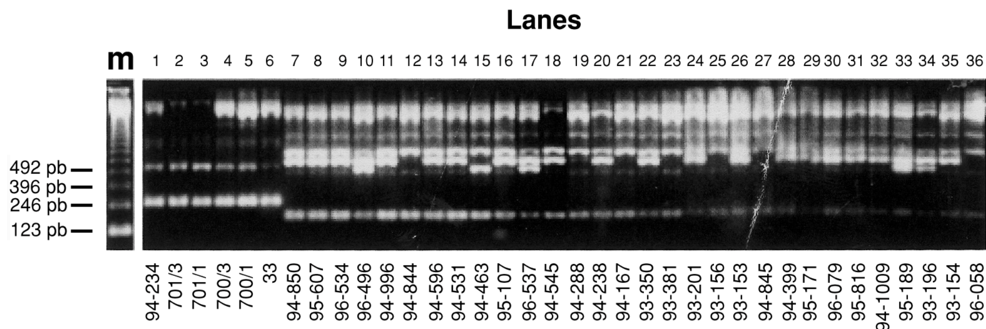

Fig. 1
RAPD fingerprinting patterns obtained withAB1.12 for *Candida dubliniensis* and *Candida albicans* clinical isolates. Lanes 1 to 6, *C. dubliniensis* isolates; Lanes 7 to 36, *C. albicans*; m, molecular weight marker –123 pb- (from ALONSO-VARGAS *et al.* (2000) with permission from authors and editor)

gent stem-loop regions (BOUCHER *et al.* 1996). The unique phylogenetic position of *C. dubliniensis* was further established by comparison of the sequences of the entire small rRNA genes (approximately 1.8 kb) of *C. dubliniensis* and *C. albicans* that revealed a difference of 1.4% (GILFILLAN *et al.* 1998).

In addition to ribosomal RNA sequences, the *ACT1* gene, which encodes the structural protein actin, has been used extensively in phylogenetic studies. DONNELLY *et al.* (1999) have amplified from a recombinant phage isolated from a genomic DNA lambda library using PCR three overlapping fragments that together span the entire *C. dubliniensis ACT1* gene (*CdACT1*). These were cloned and used to determine the contiguous sequence of the gene. Comparison of the *CdACT1* sequence with the *C. albicans* homologue (*CaACT1*) revealed that although the exons are 97.9% identical the introns are only 83.4% identical. Phylogenetic trees generated using *ACT1* exon and intron sequences from a range of yeast species unequivocally confirmed the phylogenetic position of *C. dubliniensis* as a unique taxon within the genus *Candida*. Analysis of the *ACT1*-associated intron sequences from 10 epidemiologically unrelated *C. dubliniensis* isolates from disparate geographical locations showed a very low level of intraspecies sequence variation. In order to develop an accurate and rapid method to identify *C. dubliniensis* from primary isolation plates the significant divergence between the *C. dubliniensis* and *C. albicans ACT1* intron sequences was exploited by designing *C. dubliniensis*-specific PCR primers. Using a rapid boiling method to produce template DNA directly from colonies from primary isolation plates in 10 min, these primers were used in a blind test with 122 isolates of *C. dubliniensis*, 63 isolates of *C. albicans* (including 10 isolates of *C. albicans* var. *stellatoidea*) and isolates of other clinically relevant yeast species. Only *C. dubliniensis* yielded the *C. dubliniensis*-specific 288 bp amplimer. Use of this technique on colonies suspected to be *C. dubliniensis* allows their correct identification as *C. dubliniensis* in as little as 4 h.

MEYER *et al.* (2001) have proposed PCR fingerprinting using microsatellite ([GACA](4)) and minisatellite ([5′-GAGGGTGGCGGTTCT-3′]), derived from the core-sequence of the wild-type phage M13 specific oligonucleotides as a simple, reliable and highly reproducible molecular tool to differentiate between strains of *C. albicans* and *C. dubliniensis*. *C. albicans*-specific primers have also been designed based on *PHR1* sequences that do not yield amplimers when used with *C. dubliniensis* template DNA (KURZAI *et al.* 1999). Restriction fragment length polymorphism analysis of amplimers obtained using PCR primers flanking various regions of the rRNA locus have also been demonstrated to allow the discrimination of *C. dubliniensis* from *C. albicans* (MCCULLOUGH *et al.* 1999). A PCR en-

zyme immunoassay using a *C. dubliniensis*-specific DNA probe derived from the internal transcribed spacer 2 (ITS2) region of the rRNA locus has also been developed (ELIE *et al.* 1998). PARK *et al.* (2000) have used a DNA sequence analysis of the ITS2 region of rRNA genes from reference *Candida* strains to develop molecular beacon probes for rapid identification of *C. dubliniensis* and *C. albicans*. Molecular beacons are small nucleic acid probes that brightly fluoresce when bound to their targets with the significant advantage of a higher degree of specificity. When applied to an unknown collection of 23 *C. albicans* and *C. dubliniensis* isolates, the species-specific probes were 100% accurate in identifying both species following PCR amplification of the ITS2 region.

WILLIAMS *et al.* (2001) have examined sequence variations in the ribosomal DNA (rDNA) ITS regions of *C. albicans* and *C. dubliniensis*, with a view to identifying sequence differences that would enable consistent differentiation of these two species by restriction fragment length polymorphism analysis. The ITS1 and ITS2 regions, together with the entire 5.8S rRNA gene of the strains, were amplified by PCR, using primers ITS1 and ITS4. PCR products from both species were of similar size (around 540 bp). However, sequence analysis revealed over 20 consistent base differences between the products of the two species. On the basis of sequence variation, the restriction enzyme *Msp*A1 I was selected and used to differentiate the PCR products by restriction fragment length polymorphisms analysis. *Msp*A1 I yielded two discernible fragments from *C. albicans* PCR products. *C. dubliniensis* PCR products appeared undigested, providing an approach to differentiate the two species.

POSTERARO *et al.* (2000) developed a PCR-based assay to detect and identify medically important yeasts in clinical samples. The authors used a set of primers for amplifying a fragment of the *ERG11* gene for cytochrome P-450 lanosterol 14-alpha-demethylase. The PCR product was analyzed in a reverse cross blot hybridization assay with species-specific probes directed to a target region of the *ERG11* gene of *C. albicans*, *C. dubliniensis*, of other six medically important species of *Candida*, *Saccharomyces cerevisiae*, and *Cryptococcus neoformans*. The PCR-reverse cross blot hybridization assay correctly identified multiple isolates of each species tested and no cross-hybridization was detected with any other fungal, bacteria, or human DNAs tested. Species identification time was reduced from a mean of 4 days with conventional identification to 7 h with the molecular method.

YOKOYAMA *et al.* (2000) have sequenced a 396-bp region of the mitochondrial cytochrome B gene of the most common clinically important *Candida* species, including *C. dubliniensis*. Multiple alignments of nucleotide and deduced amino acid sequences revealed species-specific nucleotides and amino acids. Nucleotide- and amino acid-based phylogenetic trees were constructed and using the database, presumptive *C. dubliniensis* identification should be possible within a working day.

A species-specific repetitive DNA element (Cd25) has been identified in *C. dubliniensis* which shows promise for use as a specific fingerprinting probe for this species and will aid in the epidemiological analysis of *C. dubliniensis* infections (JOLY *et al.* 1998). Cd25 can be a good probe since it is stable over time, is a truly *C. dubliniensis* specific probe, generates complex patterns, is distributed throughout all *C. dubliniensis* chromosomes, and has separated a worldwide collection of 57 *C. dubliniensis* isolates into two distinct groups. HANNULA *et al.* (2000) sought clonal differences among *C. albicans* and *C. dubliniensis* isolates recovered from patients with and without immunodeficiency. PCR amplification using the random sequence primer OPE-03 enabled grouping *C. dubliniensis* isolates in two genotypes (I and II) and *C. albicans* isolates in 15 genotypes (I-XV), but no significant difference was found by the authors in the distribution of genotypes between the patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy and the healthy subjects.

Despite the advantages of PCR-based methods over classic phenotypic ones, the application of molecular techniques is relatively limited due to constraints of either technical staff

training or in laboratory financing. However, PCR is being increasingly used within hospital laboratories for detection of human gene mutations, HLA typing, and viral diagnostics, and therefore it is possible that will be more widely used in the future (WILLIAMS and LEWIS 2000).

Virulence

At the cellular and molecular level, the 'commensal' *Candida* adopting a pathogenic behavior has to switch from a quiet way of life to a more complicated one to overcome the numerous human body barriers. *Candida* has to go through many steps to colonize and proliferate. Decrease in host defenses is not enough to explain invasion and infection and this fungus need a particular strategy to penetrate and grow within the human tissues (SENET 1997). Virtually nothing is known about the virulence factors of *C. dubliniensis*. However, it might be expected that this species may share the ability to produce certain putative virulence factors with *C. albicans*.

To switch from saprobe to pathogen, *Candida* develops some phenotypic characteristics, which allow adhesion and penetration. The tendency to change *Candida* behavior is very greats and in part depends on its environment. *Candida* has to overcome two main obstacles to be a successful pathogen, host mechanisms to interfere the adhesion of *Candida* to human tissues and the production of hydrolytic enzymes. Both processes are associated and related to morphological variations. The dimorphic transition in *C. albicans* from blastospore to the filamentous stage increases the adhesion and proteinase secretion. *C. dubliniensis*, as *C. albicans*, is dimorphic, but kinetics of hyphal production in *C. dubliniensis* looks slower than that observed for *C. albicans* (GILFILLAN *et al.* 1998). Hyphae are related to a higher adherence to cells and this may contribute to the apparent lower virulence of *C. dubliniensis*. But other characteristics are related to adherence and virulence in *Candida* as phenotypic switching, hydrolytic enzymes (as proteinases and phospholipases) production or hydrophobicity.

HANNULA *et al.* (2000) have observed that *C. dubliniensis* isolates exhibited high frequency phenotypic switching significantly more frequently that *C. albicans* isolates did. This fact can be considered as a favorable factor for adaptation of this species to the host environment. *C. dubliniensis* possesses homologues of seven *C. albicans* secretory aspartyl proteinase genes (*SAP*). An early study on five *C. dubliniensis* isolates suggested that these isolates produced higher levels of proteinase activity than reference isolates of *C. albicans* (MCCULLOUGH *et al.* 1995). Moreover, *C. dubliniensis* isolates were more adherent to buccal epithelial cells than the *C. albicans* strains tested (MCCULLOUGH *et al.* 1995, GILFILLAN *et al.* 1998). Secretory aspartyl proteinases have been proposed to play a role in adherence to tissue. The production of phospholipases in *C. dubliniensis* is strain-related and the amount produced shows important variations among clinical isolates and depending on the surrounding conditions (HANNULA *et al.* 2000). However, southern hybridization and PCR analyses have revealed that all *C. dubliniensis* isolates examined by BENNETT *et al.* (1998) possessed sequences homologous to *C. albicans* phospholipase C1 gene (*CAPLC1*).

Microbial adherence to mucosal surfaces is the first important step in the initiation of and invasive process in oral cavity and other human mucosae. *C. albicans*, the most adherent and pathogenic species of *Candida*, uses a diversity of mechanisms to adhere to human surfaces. The strongest mechanisms involve mannoprotein adhesins. Cell surface hydrophobicity plays also an important role in adherence by providing hydrophobic interactions that reinforce the initial bond between the fungal cell and the human surfaces (JABRA-RIZK *et al.* 2001c). *C. dubliniensis* isolates seem to have a greater adherence to buccal epithelial cells than typical *C. albicans* strains and this is related to the hydrophobic interactions that facilitate adherence (MCCULLOUGH *et al.* 1995, PEREIRO *et al.* 1997, GILFILLAN *et al.* 1998). Re-

cent transmission electron microscopy studies (JABRA-RIZK *et al.* 2001b) have shown that *C. dubliniensis*, unlike *C. albicans*, displayed a constant outer fibrillar layer independent of growth temperature. The length and arrangement of cell fibrils were consistent with those observed in a hydrophobic cell phenotype (JABRA-RIZK *et al.* 1999). These data suggest that *C. dubliniensis* exhibits constant cell surface hydrophobicity.

Cell surface hydrophobicity status of *C. albicans* involves multiple surface proteins and surface protein N-glycans. The hydrophobic surface glycoprotein CAgp38 appears to be expressed by *C. albicans* constitutively regardless of growth temperature and medium. *C. dubliniensis* expresses a 38-kDa protein that cross-reacts with the anti-CAgp38 monoclonal antibody, expression that was growth medium and temperature dependent. The anti-CAgp38 monoclonal antibody has been shown to inhibit adhesion of *C. albicans* to extracellular matrix proteins and to vascular endothelial cells. Similar bulk compositional levels of hexose, phosphate, and protein in their N-glycans were determined in both species. However, a component of the *C. albicans* N-glycan, acid-labile phosphooligomannoside, is expressed much less or negligibly by *C. dubliniensis*, and when present, the oligomannosides are predominantly less than five mannose residues in length. For *C. albicans*, the acid-labile phosphooligomannoside influences virulence and surface fibrillar conformation, which affects exposure of hydrophobic surface proteins. Given the combined role in *C. albicans* of expression of specific surface hydrophobic proteins in pathogenesis and of surface protein glycosylation on exposure of the proteins, the lack of these virulence-associated cell surface hydrophobicity entities in *C. dubliniensis* could contribute to its limited ability to cause disseminated infections (MASUOKA and HAZEN 2000, HAZEN *et al.* 2001).

Candida must traverse the overlying mucus layer in order to approximate and adhere to mucosal epithelial cells (REPENTIGNY *et al.* 2000). Binding of the *Candida* species to purified small intestinal mucin showed a close correlation with their hierarchy of virulence. Significant differences ($P < 0.05$) were found among three categories of *Candida* species adhering highly (*C. dubliniensis*, *C. tropicalis*, and *C. albicans*), moderately (*C. parapsilosis* and *C. lusitaniae*) or weakly (*C. krusei* and *C. glabrata*) to mucin. Adherence of *C. albicans* to buccal epithelial cells was quantitatively inhibited by graded concentrations of mucin. *C. albicans* may both adhere to and enzymatically degrade mucins by the action of proteases, and both properties may act to modulate *Candida* populations in the oral cavity and gastrointestinal tract.

C. dubliniensis grown at 25 or 37 °C was shown to coaggregate with the oral anaerobic bacterium *F. nucleatum* (JABRA-RIZK *et al.* 2001b). However, *C. albicans* demonstrated similar coaggregation only when hydrophobic or grown at 25 °C. Coaggregation of *Candida* cells with *F. nucleatum* is associated with a hydrophobic yeast cell surface. All *C. dubliniensis* isolates tested grown at either temperature, hydrophobic 25 °C-grown *C. albicans* isolates, unlike the 37 °C-hydrophilic *C. albicans* isolates, exhibited hydrophobic cell surface hydrophobicity levels with a microsphere assay and a maximum coaggregation with *F. nucleatum*. Coaggregation reactions between *F. nucleatum* and *Candida* species may be important in the colonization of the yeast in the oral cavity. The binding of microorganisms to each other and oral surfaces contributes to the progression of microbial infections in the oral cavity. When *C. dubliniensis* and *C. albicans* strains were grown at 37 °C on SABOURAUD dextrose agar, only *C. dubliniensis* strains coaggregated with *F. nucleatum* ATCC 49256 and no *C. albicans* strains showed coaggregation. Coaggregation at all growth temperatures was inhibited by mannose and alpha-methyl mannoside but not by EDTA or arginine. The coaggregation reaction between *F. nucleatum* and the *Candida* species involved a heat-labile component on *F. nucleatum* and a mannan-containing heat-stable receptor on the *Candida* species. KIRKPATRICK *et al.* (2000) have observed that *C. albicans* had a competitive advantage over *C. dubliniensis* in broth culture and under biofilm growing conditions. However, with the presence of a supporting structure for biofilm formation, as a

denture or the microbial dental plaque, *C. dubliniensis* was able to better withstand the competitive pressures from *C. albicans*.

Cell surface hydrophobicity also plays a critical role in adhesion of microorganisms to phagocytic cells. Hydrophobic cells of *C. albicans* have been reported to be less sensitive to phagocytic killing than hydrophilic cells. *C. dubliniensis* displays cell surface hydrophobicity at 37 °C in contrast to *C. albicans*. To elucidate this issue, PELTROCHE-LLACSAHUANGA *et al.* (2000b) determined the levels of phagocytosis, oxidative burst and killing by human neutrophils of *C. dubliniensis* compared to *C. albicans* cultured at 37 °C. Test results revealed no statistically significant differences between both species for the level of phagocytosis, evoked oxidative burst, and killing. The authors considered that human neutrophils are equally efficient against *C. dubliniensis* and *C. albicans*.

The *ALS* (agglutinin-like sequence) gene family of *C. albicans* encodes cell-surface glycoproteins implicated in adhesion of the organism to host surfaces (HOYER *et al.* 2001). Southern blot analysis with *ALS*-specific probes suggested the presence of *ALS* gene families in *C. dubliniensis* and *C. tropicalis* (three partial *ALS* genes were isolated from each organism). Phylogenetic analysis of the *ALS* and *SAP* families show that the *ALS* family is younger than the *SAP* family. *ALS* genes in *C. albicans*, *C. dubliniensis*, and *C. tropicalis* tend to be located on chromosomes that also encode genes from the *SAP* family, yet the two families have unexpectedly different evolutionary histories (HOYER *et al.* 2001).

STAIB *et al.* (2000) have developed a system for the genetic transformation of *C. dubliniensis* that is based on the use of the dominant selection marker MPA(R) from *C. albicans* that confers resistance to mycophenolic acid. Using this transformation system, a green fluorescent protein reporter gene that was genetically engineered for functional expression in *C. albicans* and placed under control of the inducible *C. albicans SAP2* promoter was integrated into the *C. dubliniensis* genome. Mycophenolic acid-resistant transformants containing the *SAP2P*-green fluorescent protein fusion fluoresced under *SAP2*-inducing conditions but not under *SAP2*-repressing conditions. These results demonstrate that the MPA(R) selection marker is useful for transformation of *C. dubliniensis* wild-type strains, that the green fluorescent protein reporter gene is functionally expressed in *C. dubliniensis*, and that the *C. albicans SAP2* promoter can be used for controlled gene expression in *C. dubliniensis*. These genetic tools will allow the dissection of the differences in virulence characteristics between the two pathogenic yeast species at the molecular level.

The only available published data from an animal model is equivocal. The *in vivo* virulence of four *C. dubliniensis* isolates and one reference *C. albicans* strain was tested in a systemic mouse model of infection. With an inoculum size of 2×10^6 cells per mouse the *C. dubliniensis* strains were clearly less virulent than the reference *C. albicans* strain. However, when the inoculum was increased to 1×10^7 cells per mouse the results were less clear-cut. These data are clearly very preliminary and are based on limited numbers of strains. In addition, a systemic infection model is not ideal for the analysis of virulence of organisms implicated in meanly superficial infections (SULLIVAN *et al.* 1999).

Antifungal susceptibility

COLEMAN *et al.* (1997a) have suggested that the recent emergence of *C. dubliniensis* as a human pathogen may have resulted from selection due to the widespread use of antifungal drug therapy. However, the great majority of *C. dubliniensis* isolates are susceptible to common (amphotericin B, fluconazole, or itraconazole) and new antifungal agents (voriconazole, caspofungin, anidulafungin, ravuconazole or posaconazole) (Table 4) (MORAN *et al.* 1997, KIRKPATRICK *et al.* 1998, ODDS *et al.* 1998, PFALLER *et al.* 1999, QUINDÓS *et al.* 2000, RUESGA *et al.* 2000).

In a recent study (PFALLER *et al.* 1999) using the NCCLS M27-A broth dilution method (1997), approximately 97% *C. dubliniensis* isolates were susceptible *in vitro* to fluconazole, the most commonly used agent in the treatment of candidiasis. Isolates with dose-dependent

Table 4
In vitro antifungal susceptibilities of *Candida dubliniensis* clinical isolates

Antifungal agent	*In vitro*	susceptibilityReference
5-fluorocytosine	Excellent	PFALLER *et al.* (1999), QUINDÓS *et al.* (2000)
Amphotericin B	Excellent	KIRKPATRICK *et al.* (1998), PFALLER *et al.* (1999), QUINDÓS *et al.* (2000)
Anidulafungin	Good	QUINDÓS *et al.* (2000)
Caspofungin	Excellent	PFALLER *et al.* (1999), RUESGA *et al.* (2000)
Clotrimazole	Excellent	TORRES-RODRÍGUEZ *et al.* (1999)
Eberconazole	Excellent	TORRES-RODRÍGUEZ *et al.* (1999)
Fluconazole	Good (Decreased)	MORAN *et al.* (1997), JABRA-RIZK *et al.* (1999), QUINDÓS *et al.* (2000), RUHNKE *et al.* (2001)
Itraconazole	Good, (Decreased)	PFALLER *et al.* (1999), QUINDÓS *et al.* (2000), RUHNKE *et al.* (2001)
Ketoconazole	Good (Decreased)	TORRES-RODRÍGUEZ *et al.* (1999), QUINDÓS *et al.* (2000)
Lipidic Amphotericin B	Excellent	QUINDÓS *et al.* (2000)
Liposomal, Nystatin	Excellent	QUINDÓS *et al.* (2000)
Posaconazole	Excellent	PFALLER *et al.* (1999), RUESGA *et al.* (2000)
Ravuconazole	Excellent	PFALLER *et al.* (1999), RUESGA *et al.* (2000)
Terbinafine	Excellent	RYDER *et al.* (1998)
Voriconazole	Excellent	KIRCKPATRICK *et al.* (1998), PFALLER *et al.* (1999), QUINDÓS *et al.* (2000)

Lipidic amphotericin B, Amphotericin B colloidal dispersion, Amphotericin B lipid complex, and Liposomal amphotericin B.

susceptibility (MIC 16–32 µg/ml) or resistance (MIC ≥ 64 µg/ml) to this azole agent has been described (KIRKPATRICK *et al.* 1998, MORAN *et al.* 1997, ODDS *et al.* 1998, QUINDÓS *et al.* 2000). The comparison of the geometric mean MICs for fluconazole, itraconazole and ketoconazole of 58 clinical isolates each of *C. albicans* and *C. dubliniensis* revealed that the MIC values of *C. dubliniensis* isolates were significantly and consistently higher than those of the *C. albicans* ones (ODDS *et al.* 1998). QUINDÓS *et al.* (2000) described four isolates resistant to fluconazole that were also resistant to ketoconazole, and three of them were also resistant to itraconazole. However, these isolates were highly susceptible to 5-fluorocystosine, amphotericin B and nystatin lipidic formulations, posaconazole and voriconazole and anidulafungin.

Moreover, the simple fact of growing colonies on agar medium containing sequentially increasing concentrations of fluconazole results in the development of resistance (MORAN *et al.* 1997). This ability of *C. dubliniensis* to generate resistant to fluconazole derivatives may be a significant factor of ecological adaptation, giving them a selective advantage in patients treated extensively with fluconazole and having therapeutic consequences in HIV-infected patients (SULLIVAN *et al.* 1999). Recently, RUHNKE *et al.* (2001) have described the case of a patient with recurrent episodes of oral candidiasis that finally suffered from fluconazole-refractory oral and esophageal candidiasis. The patient was monitored for 4 years, showing a persistent colonization by both *C. albicans* and *C. dubliniensis*. From the first episode of candidiasis, the patient was treated with fluconazole becoming unresponsive to 400 mg/day. *In vitro* susceptibility testing revealed the development of resistance to flu-

conazole in both species. Molecular typing confirmed the persistence of the same *C. albicans* and *C. dubliniensis* strains. This observation could demonstrate that *C. dubliniensis* develops resistance to fluconazole in patients repeatedly exposed to this antifungal drug.

Analysis of the resistance mechanisms in both clinical and *in vitro*-generated resistant organisms has revealed that overexpression of the major facilitator protein Mdr1p appears to be largely responsible for the resistance phenotype (MORAN *et al.* 1998) . This is in contrast to the situation in *C. albicans* where it has been suggested that overexpression of the ABC transporter protein Cdr1p is a more common mechanism of fluconazole-resistance (SANGLARD *et al.* 1995, ALBERTSON *et al.* 1996, SULLIVAN *et al.* 1999, STAIB *et al.* 2001).

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